Chapter 4

Management Practices for Insect Resistance in *Bt* Maize

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http://dx.doi.org/10.5772/61653

Abstract

The failure to reduce the population of a phytophagous species recognized as a key pest in a given situation usually occurs by not using the principles of Integrated Pest Management (IPM). The control of insect pests in agriculture has been done mainly through the application of chemical insecticides. However, chemical insecticides has lost effectiveness due to the selection of populations of resistant insects and cause adverse environmental effects. The main resistance management programs (IRM) strategy is the use of 'high dose/refuge', which involves the use of high dose of *Bt* protein in plants, promoting high mortality of heterozygotes associated with the planting of refuge, ie, a proportion of the crop in which it must be planted a non-*Bt* variety, allowing the survival of susceptible individuals. The emergence of *Bt* crops is an important step between the tactics available for pest control in various crops such as maize, canola, cotton and, in the near future, soybeans.

Keywords: Insect, Resistance, Management

1. Introduction

The history of integrated pest management in soybeans in Brazil is linked to the changing concept in pest control that occurred in the 1960s, a period when the world was alerted to the dangers of abusive use of pesticides [1, 2]. This fact prompted government policies to reduce the use of these chemicals through incentives for the adoption of integrated pest management programs in different crops. It was then that the concept of integrated management began to be popularized and is now considered a major technological breakthrough. As it is common knowledge, the IPM aims at integrating various management tactics instead of relying on the control by the exclusive use of insecticides [3]. Its concept essentially consists of a decision-making process involving the coordinated use of multiple tactics to optimize the control of all classes of pests in a sustainable and economically compatible way [4]. This philosophy of management has spread worldwide and arrived in Brazil, being rapidly incorporated into the control of pests, especially for maize crop.

Brazilian agriculture has evolved in recent years, with significant yield gains in many economically important crops, including maize. Besides the use of agricultural inputs with quality and cutting-edge technologies, the climate in general has contributed to the increase in production. Despite these favorable factors, phytophagous insects also continue to be a cause for concern in agribusiness because of its great ability to adapt to changes seen in production systems. The solution adopted to reduce the losses arising from the injury caused by pests has been, in most cases, the application of chemical insecticides. It is relatively easy to understand the reasons that have led the farmer to choose chemical control as a control tactic of "recognized" insects to cause economic losses.

The general understanding is that chemical insecticides have indisputable advantages: low cost, acts quickly, little demand in knowledge, and can be used to control various pest species. Other causes for probably using chemical control almost in a predominant way is the lack of knowledge about other control tactics or even the lack of conviction about the effectiveness of these alternative methods such as integrated pest management.

The efficiency of chemical control is often not the expected by the simple fact that it depends on several technical factors, which most often are not considered when applying the product. Sprayer type, application type, nozzles for spraying, droplet size needed for good plant cover, solution volume, application speed, climatic factors (such as wind speed, rainfall, temperature, and humidity), phenological stage of the plant and of the pest target, attack site and economic level damage of the pest, and even the applicator qualification are some factors that can compromise the action of the applied product. Therefore, when these factors are not considered, the probability of not achieving the expected results is high.

As soon as frustrations to control a certain pest begin to occur, the farmer must analyze, along with an expert, the causes of failure of the means adopted so far. When this analysis is not done, in general, there is always a risk of a mistaken decision making and probably leading to a situation worse than already detected. For example, making new applications or mixing or changing active ingredients (without considering that perhaps the causes are not related to the product used). Applications in excess also entail higher cost, with no evidence that they would be effective to control the pest satisfactorily.

2. Integrated Pest Management (IPM)

The failure to reduce the population of a phytophagous species recognized as a key pest in a given situation usually occurs by not using the principles of integrated pest management

(IPM). By its name, it is already implied that the solution of phytosanitary issues should not be thought of in a single tactic, even though apparently it may be "very practical" and convenient for the farmer. Because of lack of implementation of IPM, it is easy to understand statements and questions, such as the following: there are cases of pest resistance to insecticides; it is necessary to increase the dose of products; there are chemical residues in the soil, water, and harvested products; there are pesticides effects on flora and fauna; cost of control is prohibitive.

Brazil is already becoming a major agrochemical consumer in the world. Information of this nature in the media needs to be changed. What is expected is to reach the point of having an IPM program where there is satisfaction of both the farmer and the consumer, including the protection of the environment as a whole [5].

2.1. Fall armyworm (*Spodoptera frugiperda*) and *Helicoverpa armigera*

In maize (*Zea mays* L.), historically, fall armyworm has been the main pest. However, it is interesting to note that even with the advance in science in providing new technologies for their control, the pest is still present in the agroecosystem causing losses to agribusiness, even with use of means for their control. The lack of IPM should again be highlighted. As a main feature, the pest has as hosts several cultivated plant species or native species, available year-round in Brazil. Furthermore, general climatic conditions in the country are not limiting for their development.

As a result of this, the moth can be detected all year round in traps containing pheromone as attractive. That is, there is the real possibility of having the presence of caterpillars in their hosts just after the emergence of the plant and also throughout the plant cycle.

The moth of *S. frugiperda* lays its eggs in clusters, and each cluster contains up to 300 eggs. At hatch, the aggregate larvae begin to feed, in a short period of time, in the plant where the eggs were placed, by ragged feeding the leaf, leaving a characteristic symptom. Subsequently, they migrate into adjacent plants. In the migration process, the caterpillar produces a thread that is adhered to the leaf and is projected into the air by the wind and is easily carried to other plants. Often, in the new plant, the caterpillar goes toward the interior of the leaves still rolled ("whorl") without causing the symptom of "ragged feeding."

This symptom has been used as an indicator to control the pest. However, for the reasons mentioned, it may underestimate the level of infestation. The caterpillar phase lasts around 21 days, influenced mainly by temperature. When the initial infestation coincides with the "whorl" phase, the caterpillar remains housed in this location. When fully developed, by reaching the size between 4 and 5 cm, it leaves the whorl and heads to the ground, where it becomes known as the pupa stage. Eleven days after this stage, the adult emerges, which will restart another cycle. The period of time between laying the eggs and the appearance of a young adult insect varies between 35 and 45 days. Therefore, from the same oviposition, there could be at least three discrete generations during a maize cycle. However, because the flow of moths is constant, it normally occurs in overlapping generations, and therefore caterpillars and postures of different ages can be found in the same plant at the same time. This fact is usually a complicating factor for applying insecticides via spraying. Caterpillars of different ages require different doses of the applied insecticide, either chemical or biological. Therefore, the management of the pest is essential to know not only the pest population density but also the distribution by age-group of insects. It is not an easy task when making the decision on the need for control after sampling only based on symptoms of damage such as the scraping of leaves. A decision on the need to control based on captured moths in pheromone trap, when placed in the area soon after planting, is a more efficient procedure than those based on the injury symptom of the pest.

Because the continuous flow of *S. frugiperda* moths is not uncommon, the presence of postures and caterpillars in more developed maize plants.

However, such insect stages are not easily observed in the plant when there is no more whorl. Often the insect can be found feeding in the tassel or ear, in silks or directly in the grains, causing direct damage to yield, because any method of control via spraying is difficult to apply in these places. In addition to machines handling difficulties in the target area, there is also the difficulty of reaching the pest, protected by the leaves or the ear husks. The presence of larvae of *S. frugiperda* in the ear can be as common as corn earworm itself, the *Helicoverpa zea*. Insect infestations in the ear can cause severe damage to the farmer, as it jeopardizes the expected yield. Therefore, alternative methods should be prioritized to such pests in maize. Obviously, to reduce the population density of fall armyworm in the ear, there must be a proper pest management in the previous stages of insect development.

Morphologically, the new species is very similar to Brazilian corn earworm (*H. zea*) and also presents a very similar life cycle. However, its potential for destruction to the preferred hosts is undeniable. Due to these similarities, the problem that initially occurred in Bahia was ascribed to *H. zea*.

The oviposition is usually performed on the style stigma of maize. At hatch, the larvae consume grains in development. Secondary bacterial infections are common in the ear. The larvae can also feed on the new leaves of the whorl, from the most developed leaves and from the tassel of the plant. Mobility, polyphagia, and high reproductive rate are attributes that differentiate *H. armigera* from *H. zea*. The caterpillars are quite aggressive, occasionally carnivorous, and can be cannibals when the opportunity arises. If disturbed, they drop from the plant and wound up on the ground. Caterpillars turn into pupae within a cocoon silk, some centimeters below the soil surface. Under favorable conditions, the development cycle can be completed in just over a month. Therefore, several generations per season are possible, especially in warmer areas.

In the tropics, reproduction continues throughout the year. *H. armigera*, also called the "old world caterpillar," is usually found in parts of Europe, Asia, Africa, and Australia, while *H. zea*, the caterpillar of the "new world," is common in the Americas. The pest is more abundant in maize during the phase of "silking," when the adult female lays the egg individually on the style stigma. Adults feed on nectar or on other exudates from different plant species. The young larvae tend to feed initially on the style stigma but soon start to feed on the grain in formation. There are six larval stages, and the fully developed larva measures about 40 mm long. Third, instar caterpillars (8–13 mm long) and so on account for 90% of all food consumed (and thus its damage). Large caterpillars (above 24 mm) are the most harmful ones once they consume approximately 50% of their diet, when they are between the fifth and sixth instars. Therefore, control measures should be directed when the caterpillars are still small (less than 10 mm).

The pupa is dark brown, measuring between 14 mm and 18 mm in length, with a smooth surface, rounded, with two parallel spines on the posterior end. The moth has a wingspan between 30 and 45 mm. Females lay over a thousand eggs in her lifetime. The ease with which the pest acquires resistance to insecticides has been considered a hallmark of the species in areas where the pest usually occurs. At these locations, the development of resistance has been most extensively documented for synthetic pyrethroids, but already there is record of resistance to other groups of insecticides as carbamates and organophosphates.

The migration movements of the species could explain the resistance propagation. In regions of origin, research has shown that maize is among the preferred hosts of the pest, followed by soybeans and cotton. In Brazil, the simultaneous presence of these three crops in the same region is common, as occurred in western Bahia, the starting point of an outbreak of the pest. However, *H. armigera* can survive in more than 300 taxa of plants.

2.1.1. Control strategies

The occurrence of insects in the ear, in general, makes the management more complex. Besides the difficulty of monitoring, there is also the difficulty of reaching the pest, protected by the leaves or the ear husks. Therefore, alternative methods should be prioritized for such pests. In the specific case of the fall armyworm, one should make a proper management also during the vegetative stage of corn.

2.1.2. Biological control

The production system for maize is the pest combat, including species that attacks the ear. A first reason is the less frequent use of chemical insecticides. This fact can be explained by the use of *Bacillus thuringiensis* Berliner (*Bt*) plants, whose consequence was a significant reduction in foliar sprayers to control fall armyworm during the growing phase of the plant. Additionally, in the case of pests that attack the ears, because the caterpillars stay housed under the straw, which reduces its exposition to chemical spraying, there is greater difficulty in controlling by other methods.

The egg phase has been considered critical in the life cycle of many species of insects belonging to the order Lepidoptera. For example, to *H. armigera* always occurs a high rate and natural mortality, reaching values above 88%, mainly in the first 3 days of oviposition. Such index can reach 95%, considering the mortality of eggs and the first larval stages. Significant indexes have also been verified for *H. zea*.

Species of *Trichogramma* mainly (Hymenoptera: Trichogrammatidae) and, in a lower degree, *Telenomus* (Hymenoptera: Scelionidae) are common egg parasitoids. Among larvae parasitoids, the most common include *Cotesia* spp. and *Microplitis croceipes* (Cresson) (Hymenoptera:

Braconidae); *Campoletis* spp. (Hymenoptera: Ichneumonidae); and *Eucelatoria* spp. and *Archytas marmoratus* (Townsend) (Diptera: Tachinidae).

Considering the commercial existence and experience in releasing *Trichogramma* in Brazil and in other countries, this biological control agent is recommended for both conventional and *Bt* maize planting. The inundative release of parasitoid should be associated with the monitoring of moths in the target area.

This monitoring is carried out with traps containing synthetic sexual pheromone, specific to each type of target pest. The release of the parasitoid can be made by the distribution of card plants containing parasitized eggs near the emergence of the adult parasitoid or the direct adult release. As the parasitoid has an objective to target the pest, it can also be used in soybeans, cotton, and other crops where pests cause economic damage, regardless of the size of the cultivated area.

Obviously, one should consider that chemical insecticides required for other targets must not be applied at the same time of the release of the biological control agent.

Reduced use of chemical insecticides, through the use of applied biological control, leads to the gradual return of other biological control agents. In maize, over 100 insect species have already been described as predators of phytophagous species that feeds on both eggs to larvae. Some species prey in both the immature stage, as in the adult stage. Among the most common predators are lady beetle *Hippodamia convergens* Guerin-Meneville and *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae), lacewings such as *Chrysoperla* spp. (Neuroptera: Chrysopidae), minute pirate bugs such as *Orius* spp. (Hemiptera: Anthocoridae) and *Geocoris* spp. (Hemiptera: Lygaeidae), and earwigs such as *Doru luteipes* (Dermaptera: Forficulidae) and *Euborelia* spp. (Dermaptera: Carcinophoridae).

2.1.3. Microbial control

Viruses, bacteria, and fungi have also been used against pests of maize. Especially for *H. armigera* control, in the literature, although they mention the use of baculovirus, they highlight the increase use of *Bt*. The control efficiency with microorganism depends largely on the period and the application technique because caterpillars cannot be protected within the ear.

In Brazil, there is a great experience in using baculovirus to control fall armyworm. In other countries, a commercial product based in nuclear polyhedrosis virus (NPV) already exists, such as, for example, in the USA, to control *H. zea* and *H. armigera*. To achieve success in the control ear pests with virus, the product must be applied in order to hit the target, both for the attack location as compared to the caterpillar development stage, which cannot be greater than 10 mm in length.

Similarly to the virus, maize ear pests can be controlled by the *Bt*-based products. However, the efficiency of the products depends essentially on adjustment of the solution volume (liters of spray solution/ unit area) that can be evaluated by the use of sensitive papers which should obtain a minimum number of 30 drops cm^{-2}.

http://dx.doi.org/10.5772/61653

2.1.4. Chemical control

The same cares from microorganism applications are valid for the use of chemical insecticides. In addition to the restrictions, the possibility of a negative action of product on populations of natural enemies is considered. This fact is critical when it comes to reaching a target pest that is generally protected against the action of the applied products.

On the other hand, the exposure period of the pest to the action of the chemical is very small, and therefore, the application of pesticides must follow a strict pest monitoring system and thus avoid adverse effects on nontarget insects.

Eggs and larvae are often not sampled in corn because eggs are difficult to detect among the silks and caterpillars are generally being within the ear, making it a costly and low-precision process.

The moths, however, can be monitored by light traps and pheromone traps. Both genders are caught in light traps and only males are attracted by the pheromone. Both types of traps give an estimate of when the moths invade or emerge in a given area. However, pheromone traps are easier to use because they are selective. The pheromone is usually used in conjunction with a suitable trap, the inverted cone type, or the Delta type. Moreover, the presence of three to five moths per night is sufficient to indicate that pest control measures must be taken.

Therefore, improvements in cultural practices to maintain and enhance the impact of natural enemies represent an excellent strategy to improve the perspective for the natural biological control. Growing plants around the main crop and that attract natural enemies, such as sunflower, should be encouraged. The "trap crop" is often suggested for several species of pest, including the ear pest complex. It should, however, consider the high degree of preference of moths to lay eggs on maize in early stage of development of silks. Planting small plots of maize before the main crop can be interesting because the farmer can thus eliminate the initial infestation of the pest before their population grows enough to cause damage to the main crop.

In areas where pest populations initially develop into weeds and then disperse for major crops, the elimination of these plants by mowing or using herbicides or even applying insecticides can significantly reduce damage to neighboring crops.

2.2. Sugarcane borer (*Diatraea saccharalis*)

Indirect losses caused by this pest are more important economically because of the galleries built inside the stalk, thus the plants become more susceptible to tipping, tassel infertility, and reduced productivity and still favor the entry of opportunistic pathogens. According to EMBRAPA [6], by attacking the interior of the stalk of the plant, the larvae cause damage that can result in losses between 10% and 50% on yield. The highest losses are results from attacks in the internodes that are closer to the ear because it results in interference in the movement of nutrients produced by the plant, which are carried to a higher production of leaves instead of grain production.

The adult, with nocturnal habits, has the aspect of moth, with the forewings of a straw-yellow color, some brownish drawings, and whitish hind wings and a 25-mm wingspan. The caterpillars measure approximately 22–25 mm long, with brown head and whitish/yellowish body with numerous dark spots.

Regarding the cycle, oviposition is made in maize leaf after mating, generally on the dorsal side. The number of eggs in each oviposition is from 5 to 50, with an imbricated posture, resembling snake leather or fish scale. Immediately after hatching, and upon reaching the second instar, they enter the stem. Its attack can be identified by the inlet and outlet holes, as well as the longitudinal opening of the maize stalk, where the presence of the caterpillar or the passageway left by it is observed.

In high infestations, the attack of this insect can cause losses up to 21% in production. It can attack 65 plant species such as sugarcane, maize, millet, sweet sorghum, wheat, grain sorghum, and rice, besides many other grasses (Poaceae) and spontaneous weeds such as *Sorghum halepense*, *Paspalum* sp., *Panicum* spp., and *Holcu* ssp. Moreover, *Andropogon* ssp. The larvae damage maize in various ways: in small plants, by attacking the whorl, causing holes in the leaf blade to the death of the meristem. In more developed plants, they open galleries, feeding on the stem. These galleries are usually longitudinal but may present circular aspect, making the plant very susceptible to falling. Damages can also occur in the ear, allowing the cross infestation with weevils *Sitophilus* spp.

2.2.1. Methods of control

2.2.1.1. Chemical control

Depending on the behavior of this pest, chemical control usually does not present satisfactory result, unless the attack begins very early. In this case, seed treatment with systemic insecticides or pyrethroid sprays directed toward the base of the plant gives good results.

2.2.1.2. Biological control:

In the past 60 years, the biological control of this pest in sugarcane crop has been successful with the caterpillar parasitoid *Cotesia flavipes* and, more recently, with the egg parasitoid *Trichogramma galloi* and may be extended to the control methodology for the maize crop.

2.2.1.3. Mechanical control

Elimination of crop residues and host plants, especially grasses (Poaceae), help reduce the infestation for the next crop season.

2.3. Black cutworm (*Agrotis ipsilon*)

Black cutworm, from the genus *Agrotis*, constitute an important group of insect pests, mainly due to damages to the large number of cultivated plants and their wide geographic distribution. *Agrotis ipsilon* is the main species of black cutworm referred to in Brazil and is a polyphagous insect, which attacks mainly horticultural crops [7]. It can also attack other species of different plant families, in crops such as maize, soybeans, beans, and cotton [8].

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The adults of this pest are moths with a 35-mm wingspan, whose anterior wings are brown with some black spots, and posterior are hyaline white, with a gray lateral edge [9]. Eggs are deposited on the shoot of the plant, stalks, stems, or on the ground near host plants; they are whitish and may be found individually or in groups. Each female can lay over a thousand eggs in a lifetime [8].

After the first instar, the caterpillars are directed to the ground, where they remain protected during the day. They measure up to 5 cm in length, are robust, smooth, and in a variable coloration, with a predominance of dark gray and brown with black spots. They have nocturnal habits and are housed in the soil under debris during the day [11].

Regarding the cycle, after 4 days of the oviposture on the leaves, the caterpillars emerge. After approximately 30 days, they become pupae and remain in the soil for a period of 10 to 20 days until they become adults. The process varies 34–64 days (egg: 4; caterpillar: 20–40; pupae: 10–20). A female can lay up to 1,260 eggs, with a preoviposition period of 3 days [8].

The caterpillars attack at night, and to find them during the day, you need to revolve the soil at the base of the host plant. The main damage occurs on the establishment period of the crop when the caterpillars cut the young plants—seedlings of up to 20 cm—tumbling them and may cause high reduction of the stand. However, attacks in older plants can occur, which in this case will demonstrate the presence of cut leaves or galleries open at the stem base (they can cause the symptom of “dead heart”) or more shallow roots.

When the death of plant is not observed, the attack causes tillering. It is not common to see small caterpillars exerting plant cutting activity; they often destroy the leaf blade and the petiole [8, 9].

2.3.1. Methods of control

To have an effective system of control for this pest, we recommend the use of various tactics of control, individually or harmoniously, creating a management strategy based on cost–benefit analyzes and with a reduction on the impact on the farmers, the society, and the environment adopting IPM.

2.3.1.1. Cultural control

Early desiccation is a practice that can reduce the infestation of *Agrotis* spp. since the moths prefer to lay eggs on plants or crop residues still green. The highest incidence of attack occurs in areas of not cleaned and heavy soil. In this way, the correct postcultivation management is indispensable to keep the pest below the economic injury level [9].

2.3.1.2. Insecticide application technology

Due to the nocturnal habit of this pest, another management tactic that is important is the quality of pesticide application technology. This must be done directing the jet spray to the base of the plant, preferably in the early evening and with a high solution volume [8].

2.3.1.3. Seed treatment

Due to the nocturnal habit of the pest and the difficulty of being hit directly by pesticides, seed treatment with systemic insecticides can be very effective to control this pest. This practice has shown to be even more efficient in areas with history of high occurrence and recurrence.

2.3.1.4. Chemical control

As an emergency control, chlorpyrifos can be used in spraying, preferably in the early evening.

2.3.1.5. Biotechnology

The use of genetically modified seeds with insecticidal proteins can be a tool to control this pest but is more effective to control small caterpillars [8].

2.4. Cornstalk borer (*Elasmopalpus lignosellus*)

It is very difficult to manage cornstalk borer in sandy soils (well drained) and under cerrado vegetation (savannah) (especially in the first year of cultivation) in dry periods with high temperatures, in particular in the first 30 days after emergence. Just as the black cutworm, the cornstalk borer causes damage also known as "dead heart" and causes significant losses in the stand.

The moth of nocturnal habits has a 1.5- to 2.5-cm wingspan and has gray-yellowish wings. It lays eggs preferably in the base of plants or in the soil, which are initially clear, but with the approach of the hatching become dark red. The caterpillar has blue-green color, with brown, purple, or dark brown transverse stripes, and measures about 1.5 cm [8].

It is a sporadic pest, however, polyphagous; it feeds from diverse crops (such as soybeans, maize, and cotton), with great capacity for destruction in a short period of time, especially between VE and V3 stages. After hatching, the caterpillar scrapes the plant leaves and starts its penetration in the stem remaining in this location during the day. It builds a shelter with web and dirt, which is attached to the gallery's opening also made by it, where droppings are being accumulated. Its damages are associated with drought after plant emergence, and the greatest damages are observed in conventionally tilled fields, with light, well-drained soil, and lower damages in sites with tillage and irrigation.

In maize, it feeds inside the stem and goes upward toward the growing point of the plant (apical bud), eventually damaging it, causing reduction in size or even death of the youngest leaves, a symptom known as "dead heart." In certain situations, the attack symptoms of cornstalk borer do not necessarily cause the dead heart but shoots at the base of the plant and present symptoms very similar to the attack of green belly stink bug (*Dichelops* spp.).

In soybeans and cotton, cornstalk borer feeds on the stem and branches of seedlings, causing wilting, drying, tipping, and even death. In larger plants, the pest opens galleries inside the stem. The damage is greater when the attack occurs early in the development of culture, when the young plants are eaten and have less ability to recover. During the larval stage, the insects

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are highly mobile and can migrate from dead plants to live ones and can cause major damage and even failure in planting lines. They also cause drying and death of plants, necessitating replanting [8].

2.4.1. Methods of control

2.4.1.1. Chemical control

Can be accomplished by seed treatment with systemic insecticides. Insecticides applied soon after the appearing of the pest have not shown satisfactory results, making the best option the preventive control.

2.4.1.2. Cultural control

In regions with high incidence of pest, increased seed density per area may be an alternative. Maintaining humidity also contributes to the decrease of the attack of this pest [8].

2.5. Corn earworm (*H. zea*) and (*H. armigera*)

Due to the moth habit of depositing eggs on the plant stigma and the caterpillar developing inside the ear, *H. zea* is called corn earworm.

It has pronounced larval movement in different crops and is aggressive when touched, adopting a defensive posture. The pupal development occurs in the soil and can occur optional diapause depending on weather conditions.

H. armigera has a higher attack spectrum than *H. zea*. In addition to maize, cotton, soybean, and tomato crops, the preferred targets of *H. zea*, it also attacks beans and sorghum, which causes damages to vegetative and reproductive structures.

Caterpillars of *Helicoverpa* spp. perform predation of other species of caterpillars and also on the same species (cannibalism) [8].

They have a high fertility rate and can occur up to 11 generations of the pest, with night oviposition preferably and capacity of laying 2,200–3,000 eggs on host plants, but with no predilection for specific parts of the plant [8].

For this reason, it feeds inordinately of all plant structures at an early stage, with preference for the reproductive structures in final stages of development [8].

2.5.1. Methods of control

One of the key points for success in controlling *H. armigera* and *H. zea* is to correctly identify the pest in the field, mainly due to its similarity to *Heliothis virescens*, the tobacco budworm.

It presents different behavior in relation to this pest, with aggression and resistance to insecticides based on synthetic pyrethroid characteristics [11], the joint use of agricultural practices and the integrated management of pests in a correct manner are essential.

2.5.2. Integrated pest management

The use of integrated agronomic systems, combining knowledge of the target pest, the constant monitoring of the crops that are in the system, and the adoption of practices that aimed cultural control and biological maintenance, combined with the use of biotechnologies to fight pest, are suitable forms of maintenance and control of *Helicoverpa* spp.

2.5.3. Chemical control

The use of insecticides from the chemical group diamides has shown satisfactory control in the fight against the pest.

2.5.4. Adoption of Bt maize is occurring rapidly

With only 6 years of the release of its cultivation by CTNBio, over 70% of the Brazilian maize crops were coming from transgenic crops, and it is projected to increase to 81%, which represents the cultivation area with intensive use of technology [12].

2.6. Corn rootworm (*Diabrotica speciosa*)

Among the six species of *Diabrotica* occurring in the tropics, *Diabrotica speciosa* is distinguished by economic importance to maize crops. This species is a polyphagous pest widely distributed in Brazilian states and in some countries in South America. The adults damage the shoots of various crops such as horticultural crops (solanaceous, cucurbits, crucifers plants), beans, soybeans, sunflower, and maize, causing defoliation and in some cases are vectors of pathogens. When adults feed, it transmits numerous viruses to plants. The viruses are easily transmitted mechanically and produce highly antigenic responses. The transmission of the virus from one insect to another is associated with the contact to the regurgitated material, defecated or through contaminated hemolymph. In the order Coleoptera, species of *Cerotoma* and *Diabrotica* genres are the most important vectors of viruses in the Americas. The larva has been considered one of the most important underground pests of crops such as maize, wheat, other cereals, and potato. The economical loss caused by the larva for these crops has been significant in the southern states and in some areas of the Southeast and Midwest. In the South, areas where soils are usually rich in organic matter and retain higher humidity favors the biology of larvae. In irrigated areas of the Southeast and Midwest, where several host crops are grown in succession, the damage has been representative. The larvae feed on the roots, reducing the plant's ability to absorb water and nutrients, making them less productive and subject to lodging, causing losses when harvesting is performed mechanically. For the maize crop, losses have been reported in the yield varying between 10% and 13% due to the attack, when high infestation of this pest occurs [13].

The adults are greenish color presenting three yellow spots on each shard, black tibia and tarsus and brown head, being called "patriot." They measure about 6 mm in length. Males are smaller than females. Adult longevity, the pace of oviposition and fertility depend on the type of food they feed on in the larval and adult stages.

http://dx.doi.org/10.5772/61653

The longevity may vary from 41.8 to 55.5 days for the males and from 51.6 to 58.5 days for the females. The oviposition is held in the soil around the plants. The eggs are yellow and measure 0.5 mm in diameter. The incubation period ranges from 6 to 8 days. The larva phase goes through three instars, and the larvae reaches 10 mm long, with whitish coloring, brown head, and a chitinized dark plate in the last abdominal segment. The average larval period is 18 days. The prepupa average period is 5 days and pupal period is 7 days. The life cycle varies from 24 to 40 days. The temperature is a climate factor that affects the rate of development of the immature stages as well as the longevity of adults and reproduction [13].

2.6.1. Methods of control

Chemical control has been the most widely used method for controlling various species of *Diabrotica*. In Brazil, research works about the control of *D. speciosa* larvae attacking maize crop are scarce, complicating the recommendation of insecticides and the application method to control this pest, while in other countries, information about the control of other species of the genus is abundant.

The persistence of insecticides has been considered an important factor in the control of *Diabrotica* larvae. Ideally, the pesticide persists in the soil for 6 to 10 weeks, providing protection to the plant in the most susceptible period to pest [14]. As a result, treatment of seeds with insecticides has shown problems in the control of the larvae. The use of granular insecticides or spraying in the planting groove is effective alternatives to control the larvae [13].

Biological control is a promising tactic for managing this pest. Several natural enemies are described attacking adults and larvae of *D. speciosa*. The ones with most frequent occurrence are *Celatoria bosqi* (Dip., Tachinidae), *Centistes gasseni* (Hym., Braconidae), fungi *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces lilacinus*. The control of larvae, especially with fungi, has great potential to be implemented in field conditions. As a strategy for the use of cultural control, it is important to consider that soil moisture and preparation method can affect the population of larvae. Adults have a clear preference for oviposition in darker soils with higher organic matter levels and moisture [13].

3. Insect resistance management to the *Bt* technology

The control of insect pests in agriculture has been done mainly through the application of chemical insecticides. However, chemical insecticides have lost effectiveness due to the selection of populations of resistant insects and cause adverse environmental effects.

In this context, the biological insecticide *B. thuringiensis* (*Bt*) has emerged as an alternative for the control of insect pests of agriculture. The Cry proteins produced by *Bt* have demonstrated a high specificity, and there is no evidence that directly affect natural enemies [15] as well as vertebrates [16]. These features have made the development of transgenic plants producing Cry proteins in its solubilized form possible, which give the property of resistance to insect pests. In the sequence, we will discourse about these proteins, as they are the mechanisms of action in the target insect, and their most important applications.

Bt is a gram-positive bacteria, strictly aerobic, which during its life cycle has two main stages: vegetative growth, which bacteria replicate by splitting, and sporulation, which is differentiating bacteria in the spore. *Bt* is considered a ubiquitous bacteria since it has been isolated from around the world in many different systems, such as soil, water, plant leaves, and dead insects, among others. In the sporulation phase, *Bt* bacterium is characterized by producing a parasporal body known as "crystal," which is a protein nature and has insecticidal properties. The crystal protein is formed by proteins called δ-endotoxins, also known as Cry or Cyt proteins. δ-Endotoxin proteins have been found active against insects of Lepidoptera, Coleoptera, Diptera, Hymenoptera (ants), and also against other invertebrates such as nematodes, flatworms, and protozoans.

As mentioned, there are two types of δ-endotoxins: Cry and Cyt proteins. So far, more than 733 Cry genes and 38 different Cyt genes have been cloned and sequenced [17]. This is certainly a valuable arsenal for insect pest control. The nomenclature of δ-endotoxin is based solely on the similarity of the primary sequence. By definition, any parasporal protein that presents any toxic effect on body verified by bioassay or any protein that presents similarities with the Cry proteins are considered a Cry protein. Currently, Cry proteins have been found in other species of bacteria such as *Clostridium bifermentans* (classified as Cry16A and Cry17A) with activity to mosquitoes. The Cyt are *Bt* parasporal proteins that exhibit hemolytic activity.

Cry proteins are sorted and divided into 73 groups and several subgroups, and Cyt proteins into two different groups and subgroups, based on the similarity of the amino acid sequence. The Arabic numeral designates an identity of 45% (for example, Cry1, Cry2, etc.), the capital letter corresponds to 45–78% identity (cry1A, cry1B, etc.), the lowercase letter corresponds to the identities of 78–95% (Cry1Aa, Cry1Ab, Cry1Ac, etc.), and the Arabic numeral at the end of the nomenclature indicates more than 95% identity (Cry1Aa1, Cry1Aa2, etc.).

The symptoms observed in susceptible insect larvae when *Bt* crystals and spores are ingested are as follows: cessation of intake, intestinal paralysis, diarrhea, complete paralysis, and eventually death. In general, it is accepted that the Cry proteins are forming pores, which cause an osmotic imbalance in epithelial cells since proteins bind to receptors of the cell surface digestive system.

The Cry proteins are produced as a protoxin that needs to be proteolytically processed by proteases present in the gut of susceptible insects. This proteolytic processing releases toxic fragments to the insect (protein in the solubilized form), with a mass between 55 and 65 kDa, which interact with receptor proteins present in the microvilli of intestinal cells of the target insect. Subsequently, the proteins bind to the intestinal membrane forming a lytic pore.

Despite low similarity of Cry proteins, in some cases less than 25%, these have a similar structure composed of three domains. The domain I, composed of seven α and amphipathic antiparallel helices, where six of them surrounds the helix $\alpha5$. This is the domain that forms the ion pore. Domain II consists of three folded β-sheet and three handles, where the most structural difference is observed. This is the domain less conserved among Cry proteins. However, its sequence and tertiary structure play an important role in the specificity of the protein since the handles interact with the receiver located in the microvilli of the midgut epithelial cells. Domain III consists of two antiparallel β folded sheets forming a sandwich and is also involved in the interaction with receptors.

Commercial name	Events	Protein	Applicant	Year of approval
YieldGard*	MON810	Cry1Ab	Monsanto	2007
TL**	*Bt*	Cry1Ab PAT	Syngenta	2007
Herculex**	TC1507	Cry1F PAT	DuPont and Dow AgroSciences	2008
YR YieldGard/RR2**	NK603 and MON810	CP4-EPSPS Cry1Ab	Monsanto	2009
TL/TG**	*Bt*11 and GA21	Cry1Ab PAT mEPSPS	Syngenta	2009
Agrisure Viptera*	MIR162	VIP 3Aa20	Syngenta	2009
HR Herculex/RR2**	TC1507 and NK603	Cry1F PAT CP-4EPSPS	DuPont	2009
VTPRO*	MON89034	Cry1A.105 Cry2Ab2	Monsanto	2009
TL TG Viptera**	*Bt*11, MIR162, and GA21	Cry1Ab VIP3Aa20 mEPSPS	Syngenta	2010
VTPRO2**	MON89034 7 NK603	Cry1A.105 Cry2Ab2 CP4-EPSPS	Monsanto	2010
Optimum Intrasect RR2**	MON810, TC1507, and NK603	Cry1A.105 Cry2Ab2 Cry1F PAT CP4-EPSPS	DuPont	2010
Optimum Intrasect**	TC1507 and MON810	Cry1F Cry1Ab PAT	DuPont	2011
VTPRO3**	MON89034 and MON88017	Cry1A.105 Cry2Ab2 Cry3Bb1 CP4-EPSPS	Monsanto	2011
Herculex XTRA maize	TC1507 x DAS-59122-7	Cry1F PAT Cry34Ab1 Cry35Ab1	DuPont and Dow AgroSciences	2013

*Insect resistant.
**Insect resistant and herbicide tolerant.
Source: CTNBio [20].

Table 1. General summary of maize plants genetically modified approved for marketing in Brazil.

The aminopeptidase N (APN) is a protein from the family of cadherins (BtR) and have been proposed as potential recipient of Cry1A proteins in Lepidoptera. The APN is a protein with an apparent mass of 120 kDa, which is anchored to the membrane via a glycosylphosphatidyl group inositol (GPI). There is evidence that the interaction of the protein with the cadherin receptor promotes an additional cut in the extreme amino terminus of the Cry protein by facilitating the formation of an oligomer or "pre-poro" formed by four monomers, which is responsible for membrane insertion and pore formation. For the "pre-poro" to be inserted in the membrane, it is necessary to interact with the APN receptor. The proteins anchored in the membrane by GPI are preferably distributed in specific regions of the membrane, known as lipid rafts, which have specific characteristics due to the high content of cholesterol and glycolipids. The interaction of the Cry protein of the "pre-pore" with the APN facilitates the insertion of oligomer in the lipid rafts on the membrane, resulting in pore formation [18].

The *Bt* technology relies on the transfer and expression of resistance genes to insect pest in maize, isolated from the bacteria *B. thuringiensis* Berlinger (*Bt*) [19]. The preservation of susceptibility to *Bt* toxins in pest populations depends on resistance management programs (IRM). Table 1 presents a summary of the most important technologies for maize crop.

3.1. Considerations about the refuge area

The main IRM strategy is the use of "high dose/refuge," which involves the use of high dose of *Bt* protein in plants, promoting high mortality of heterozygotes associated with the planting of refuge, i.e., a proportion of the crop in which it must be planted a non-*Bt* variety, allowing the survival of susceptible individuals to mate with possible resistant ones [21]. A protein may have high dose activity for a pest species and moderate or low dose to others, which does not impair the IRM because it is expected a simultaneous action of other mortality factors, such as natural enemies [22]. In this scenario, the adoption of the refuge area is also key to the IRM.

The explanation for cases of resistance to Bt crops appears to be related to the nonuse of high dose/refuge [23] strategy, particularly the nonadoption of refuge [24, 25].

The configuration of refuge areas may vary, but basic criteria of size and proximity to the *Bt* crops based on the target pest bioecology should be followed [22] so that these areas produce consistent proportions of adults for mating and maintaining susceptibility. In Figure 1, specified examples of refuge areas settings are shown.

Figure 1. Examples of refuge areas settings.

A good example of an alternative method for pest control, especially in Brazil, against fall armyworm, was the development and release of genetically modified plants such as *Bt* maize, a technology adopted with incredible rapidity in Brazil. Unfortunately, used without proper care, there are already complaints from different parts of the country in a few years of use about the presence of caterpillars and their damage above expectations. In fact, the expectation of farmers is that there would be no injuries from this pest in the crop. For fear of having economic losses, the chemical control so far left as low priority is back to be used in some areas of higher incidence even in *Bt* maize. Therefore, the importance of the technology should always be emphasized, however, pointing out that it alone will not solve the numerous phytosanitary problems in maize or other crops. For various reasons, since the commercial release of *Bt* maize, there was already a concern for the proper management of the technology to prevent breakdown of resistance by target pests. All good agricultural practices generally conveyed along with the acquisition of the seed must be strictly followed. Such practices include adopting refuge areas.

Until 2007, the scenario of maize crop in Brazil was of growing losses by caterpillar's attacks. Problems with fall armyworm, black cutworm, corn earworm, cornstalk borer, and sugarcane borer increasingly frightened the farmer, who had little efficiency in the control of these pests using insecticides.

Quickly, the *Bt* technology in maize significantly reduced the problems with chewing insects, causing the erroneous impression that the technology was "bulletproof," meaning that nothing needed to be done and that all IPM practices could be left aside. However, with passing time and the intensive use of technology, the problems with insect resistance began to appear.

Resistance can be defined as a biological and evolutionary phenomenon that occurs in response to selection pressure exerted by the different control agents.

The evolution of resistance consists in the selection of resistant individuals that are naturally present in nature, leading to increased frequency of these individuals or their genes in the pest population, leading eventually to restrictions control agent efficiency. Unlike foliar insecticides, the *Bt* crops carry a much higher selection pressure on populations of insect pests that are target to control due to continued expression of insecticidal toxins over the crop growth period. This causes a higher risk of pest developing resistance to *Bt* technology.

The continuous expression of insecticidal proteins throughout the cycle of *Bt* plants and this rapid adoption represent threats to its durability, the strong selection pressure on the pest insects [23, 26]. Indeed, cases of resistance to *Bt* toxins have been reported for maize pests such as *S. frugiperda* [27–29] and *Diabrotica virgifera* [24].

According to Kumar et al. [30], the use of refuge areas, represented by planting susceptible varieties surrounding soybean crops sown with *Bt* varieties, is the main strategy to prevent the development of resistance.

Although avoiding the phenomenon of resistance of pests to insecticides (chemical or biological) should be a constant concern in the case of *Bt* crops, the recommended strategy involves actions that require time and use of machines, which may result in hatred of farmers to compliance, resulting in lower lifetime varieties with this feature.

The aggravating factor is that due to the characteristic (or genetic) of resistance when we start to see damage in the field in a technology with medium-high dose, the frequency of alleles is now probably around 10%, with the chance that, with continuous exposure to technology, the population will be at a much higher proportion of resistant individuals in a few generations.

Since the launch of the first *Bts*, several companies warned that the IPM practices should not be set aside and, especially, the refuge area should be established in all farms. The refuge, which is the planting of at least 10% of the area with a non-*Bt* hybrid maize, allows the survival of insects susceptible to *Bt* technology. The preservation of these susceptible insects allows the crossing with possible resistant insects, resulting in a progeny of susceptible insects.

However, few farmers planted the refuge area, and when they did, spraying in such areas were constant in order to obtain the productivity in the area. The result was that even when present, in many cases, the refuge areas did not work effectively in the maintenance of susceptible insects that would mate with any resistant insect coming from the *Bt* areas. In the absence of susceptible insects from the refuge area, any surviving insects resistant to exposure to *Bt* mated with each other, allowing relatively rapid increase of the resistance alleles and increased amounts of resistant individuals in the field.

Now that the resistance break of fall armyworm to Cry1F technologies is a reality, the question is, Is the refuge still necessary and beneficial for this technology?

The answer is certainly yes, because there are other pests that are controlled by the Cry1F protein as the sugarcane borer; other insects are likely to also develop resistance in case the best management practices are not applied, and in case there is no maintenance of susceptible individuals by adopting the structured refuge. The refuge is essential to maintain the efficiency of this control. In addition, all the technologies in the market today will have their increased durability and benefit from the adoption of best management practices and refuge areas for planting.

Knowing that the refuge areas are part of the IPM and the insect resistance management, how should we proceed to make the correct use?

As previously mentioned, poor adherence of refuge use or the many insecticide applications in the refuge, eliminating susceptible individuals, resulted in an ineffective resistance management system, which favored a faster resistance evolution rate.

It is known that only the adoption of refuge is not enough to maintain the effectiveness of the technology and should also be considered to manage the use of insecticides in agriculture. The refuge should be as a donor area of susceptible insects so that they can mate with any resistant insects and the result is susceptible individuals in larger quantities. Therefore, it is necessary to maintain differential applications between the refuge and *Bt* crop so that the application rate of insecticide in the refuge should be lower than in the fields. Basically, we have to think of resistance management in *Bt* area and management of economic damage in the refuge area.

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3.2. Early desiccation followed by insecticide

The previous crops as well as weeds and volunteer plants in the environment can host the main pests that attack maize in the initial phase, influencing the predominant species and the initial pressure of pests. Thus, in the no-tillage system, pest pressure in the early stage of the crop can be greater when compared to conventional tillage.

In the case of the presence of pests in the area, it is recommended that the application of insecticide be followed by preplanting desiccation, aiming the reduction of the initial population of pests, which are the most challenging for seed treatment; the control of resident caterpillars in later instars, which can cause early damage even in *Bt* maize crops; and the maintenance of the initial stand of the crop.

Regarding the early cover crop desiccation, it aims to provide dry straw on the ground, facilitating the operation of planting and promoting the protection of the soil. The optimal timing of herbicide applications may vary according to weather conditions and the cropping system used.

It is recommended to make two herbicide application; in the first period of approximately 30 days before planting, thus avoiding the presence of green mass at the time of sowing, and in the second desiccation shortly before planting in order to control the first flow of weeds after the first desiccation.

We highlight some benefits of desiccation performed at the right moment: more efficient use of insecticide in the second desiccation, as the green cover reduces its intensity with the first desiccation (eliminating the umbrella effect for insecticide); better plantability: easier cut of the straw by planter; availability of dry straw in the crop germination period: protection of soil moisture; reduction of possible allelopathic effects of the previous crop as the main crop; and ease in weed control in the postemergency phase of the crop, if necessary.

3.3. Weed control

Some weeds may host insect pests of succeeding crops, allowing a significant amount to survive in the areas of cultivation in the off-season period. In addition, weeds can be sources of caterpillars in later instars, which presents major difficulty to control by the *Bt* technology. Some practices may contribute to a better control of weeds, as well as prevent resistance to herbicides:

- Do not leave fallow areas: use integrated practices of weed management during the year, focusing on the handling of the seed bank (crop rotation and covers).
- Start growing in clean area: make an effective control early in the preplanting and, if necessary, use a preemergent in high pressure areas of weed.
- Use the dose and the correct moment of the application of products in good management system, in compliance with the best application conditions.

- Use the postharvest management: use the association of herbicides with different modes of action.
- Monitor the results of the implemented management strategy, preventing the establishment of remnant populations of weed in the crop.
- Use the best agronomic practices to maximize crop competitiveness with weeds, also avoiding seed dispersal by agricultural implements.

Regarding the management of volunteer plants after the maize crop, it is common the occurrence of germinação of remaining grains from previous crop spontaneously;

The amount and timing of germination of these maize kernels, producing crop residues (also known as "tigueras"), depends on many factors, being the quality of the previous harvest one of the most important; herbicides called graminicides are the main management tool of these plants. Volunteer plants are controlled until the V3/V4 stage to obtain consistent and quick controls. Weed competition is prevented with subsequent soybean crop, making the early management of volunteer plants.

3.4. Seed treatment

Seed treatment (ST) is a practice that seeks control of underground and initial culture pests, a period of great susceptibility to pests. The damage caused by these pests results in crop failures due to the attack on the seeds after planting, damage to roots after germination, and shoots of newly emerged plants. The correct choice of chemical is essential to the success of this operation. We recommend using products from broad spectrum to provide efficient control of the initial pests of the crop complex, which will bring results as the protection of plants in the initial development phase, broad-spectrum pest control, and maintenance of the initial stand of the crop.

3.5. Crop rotation

Crop rotation consists of alternating the planting of different species of crops in the same agricultural area. The choice of species for crop rotation should take into account economic factors, pests, diseases, and fertilization, among others.

To obtain maximum efficiency, improving productivity capacity of the soil, the planning of crop rotation must consider, preferably commercial plants and, whenever possible, involving species that produce large amounts of biomass and rapid development, cultivated singly or intercropped with commercial crops.

Among the benefits of crop rotation in pest management in *Bt* maize, the highlights are as follows: improved physical and chemical properties of the soil, reduction of disease inoculum source for subsequent crops, reduction of the initial population of some insect pests of the crop, aid in weed management, and ability to switch herbicides for the control and increase in the system productivity.

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4. New sources of resistance

The interaction of plant-herbivore insects occurs in various combinations of genotypes and environments, which makes its coevolution process broad and diverse. For this reason, plants and insects can provide a wide range of mechanisms, which make them resistant to attack or able to circumvent the acquired resistance. Thus, when considering the coevolution as a dynamic process, we must be sure that the natural resistance or artificially acquired by an organism may be short-lived or long-lasting, but difficultly can occur permanently. On the other hand, the duration of plant resistance will be greater as lower the speed on the evolution of resistance in the insect target, in other words, we must focus on strategies to reduce the selection pressure on the target. It is precisely in this aspect that the search for new genes that may confer resistance to insects fits. For example, using more than one resistance gene in a genetically modified plant, it is possible to prolong the emergence of resistant individuals, especially if these genes relate to different sources of resistance as a toxin and a compound that attracts a natural enemy target.

The prospect of important genes in plant–insect interaction has the fundamental objective of assisting in the preparation of new alternatives, both with the identification of genes that make plants resistant or susceptible to insect attack, as with the identification of genes that are associated with the insect's ability on circumvent the defenses of their hosts. Knowledge of the physiology of insects resistant to *Bt* toxins, for example, is important to the discovery of new targets (genes or genetic polymorphisms).

Otherwise, other *Bt* toxin proteins or other natural enemies of herbivorous insects may also represent new alternatives resistance.

In this sense, studies aiming at prospecting for new important genes in plant-herbivore insect interactions can concentrate on the plant by identifying mRNA expressed (transcriptome) [31], proteins (proteomics) [32], or metabolites (metabolomics) synthesized in specific tissues and moments of the interaction, or they may focus on the insect by the use of the same tools applied to tissues or moments fundamental to the success of interaction, such as the study of the digestive proteins secreted in the midgut and that enable herbivores [31] or the study of regulatory elements of metamorphosis [34]. Alternatively, prospecting studies can focus on the interaction of model organisms for which there is already high amount of generated knowledge (genomic knowledge and tools to produce genetic alterations), such as *Arabidopsis–Scaptomyza flava* interaction (*Drosophila*) [35], or may focus on a single study or specific response mechanism by, for example, the application of a compound that is known to cause a direct defense response in plants [36].

Different strategies can be useful for gene prospecting, including comparative analyzes of transcriptoma, proteomics, metabolomics, and the functional study of genes by mutagenesis, overexpression, and gene silencing. Indeed, comparative analyzes can be exploited as ideal strategies for global exploration of important genes in plant–insect interaction. Such analyzes can be conducted in order to compare important genes in plant–insect interaction in different environmental conditions [37] in resistant and susceptible plants [38] in injured plants by different insects [39] and others.

Global prospection strategies achieved particular prominence with the use of new technologies of DNA sequencing to characterize transcriptoma (RNA-seq). With RNA-seq strategies, it is possible to generate billion bases of information in single runs (at a much lower cost than Sanger sequencing), which allows access to regulatory genes, represented by one or a few mRNAs [40] and covering full-length cDNAs [41].

Although the global strategies of gene prospecting are potentially unlimited, the success of identifying real candidates depends on the development of an efficient experimental design. On the work of Li et al. [37], the defense mechanisms of two soybean varieties, that is, resistant and susceptible to an aphid, were studied using microarrangements of cDNA, and the collection period after an infection determined by the time necessary to the insect reaches the xylem vessel elements in the plant, about 8 hours in the resistant cultivar and 3.5 hours in the susceptible cultivar.

The large-scale study of metabolites produced by plants in the presence of insect pests also consists in an innovative possibility of seeking alternatives for its control and the identification of genes or important metabolic pathways. In soybean leaves [42], it was observed that constitutively leave extracts of PI 227687 contain the isoflavonoid genistein and seven flavonol glycosides, including rutin [43], by studying the leaf extract resistant to insect genotypes PI 274454, "IAC-100," and PI 229358, which identified and quantified the flavonol rutin and the isoflavonoid genistein.

Their identification and their role in the interactions of insects with soybean plants can guide geneticists in order to keep them in descendant generations as part of the defense armory of plants against herbivores. To study if the insect resistance of genotypes PI 227687, PI 274454, and "IAC 100" is due to chemicals present in their constitution, they used extracts of these genotypes mixed to artificial diet. By the results obtained, Piubelli et al. [43, 44] found that those strata negatively affect the biology of *Anticarsia gemmatalis*. Additionally, studies have shown that the flavonol rutin causes antibiosis in *Trichoplusia ni* (Hübner) [45, 46].

In general, although the *Bt* strategy to control lepidopteran still is the world's most important in controlling pests, new sources of resistance may operate independently or may also be added to the *Bt* strategy so as to promote their own maintenance of *Bt* resistance in commercialized transgenic plants.

Molecular biology tools have supplemented the information generated by morphological and behavior studies, contributing to the elucidation of issues in the fields of taxonomy, ecology, pests population genetics, parasitoids, predators, and entomopathogenic bacteria. Its resolving power has allowed increased knowledge on the occurrence of cryptic species, differentiation of insect races, and separation of microorganisms species indistinguishable by morphological characters. These tools also have wide application in genetic studies of resistance to insecticides and toxins and in the determination of genes associated with these phenomena. On the other hand, they have facilitated the breeding works to plant resistance to insects, as well as the transformation of the beneficial organisms to increase pest control potential. Considering its potential and reducing reagent costs and simplifying processes, we expect a growing application in basic and applied fields of entomology and its related areas.

http://dx.doi.org/10.5772/61653

5. Final considerations

The first challenge will be to develop innovative formulas of the application of integrated pest management concepts that are adequate with the new and dynamic field reality, including the prevalence of tropical regions for soybean cultivation, its integration into more complex production systems and large overlap of common pests to different crops in the same system, and the great extension of crops. Framing this set is a phenomenon that has grown in importance over the past decade, greatly worrying farmers, which is the lack of manpower available for the labors on the field.

Developing biological control technologies that are technically feasible and economically competitive will also be a challenge, given the diversity of the production system pests and the impact that other forms of control, especially insecticides and fungicides, will have on biological control agents.

The emergence of *Bt* crops is an important step between the tactics available for pest control in various crops such as maize, canola, cotton, and, in the near future, soybeans. In addition to controlling some important species of Lepidoptera, a positive externality of the use of *Bt* crops will be the preservation of insects that act as predators or parasitoids of pests due to less use of insecticides to control caterpillars, nonselective to these biological control agents. However, there is the ever present risk of emergence of Lepidoptera populations insensitive to the toxin produced by *Bt* crops due to nonuse of refuge by farmers. The events are similar for different crops with *Bt* cultivars or varieties, and some pests attack more than one crop for which there are *Bt* events, increasing the risk of emergence of insensitive populations.

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References

[1] Carson, R.L. Silent spring. Boston: Houghton Mifftlin Co., 1962. 368p.

[2] Van Den Bosch, R. The pesticide conspiracy. New York: Doubleday & Co. Inc., 1978. 212p.

[3] Kogan, M. Integrated pest management: historical perspectives and contemporary developments. Annual Review of Entomology, v. 43, p. 243–270, 1998.

[4] Prokopy, R.J.; Kogan, M. Integrated pest management. In: Resh, V. H.; Cardé, R.T. (Ed.). Encyclopedia of Insects. New York, Academic Press, 2003, p. 4–9.

[5] Cruz, I. Circular encartado na revista Cultivar Grandes Culturas. Caderno Técnico, Ed. 168, 12p. 2013.

[6] EMBRAPA—Empresa Brasileira de Pesquisa Agropecuária. A Broca da Cana-de-Açúcar, *Diatraea saccharalis*, em Milho, no Brasil. Sete lagoas: Embrapa milho e sorgo, 2007. (Circular Técnica 90).

[7] Bento, F. M. M.; Magro, S. R.; Fortes, P.; Zério, N. G.; Parra, J. R. P. Biologia e tabela de vida de fertilidade de *Agrotis ipsilon* em dieta artificial. Pesquisa Agropecuária Brasileira, v. 42, n. 10, p.1369–1372, 2007.

[8] Schneider, et al. Manual de pragas do milho, soja e algodão.Sementes Agroceres, 2013.148p.

[9] Gallo, D.; Nakano, O.; Silveira Neto, S.; Carvalho, R. P. L.; Baptista, G. C.; Berti Filho, E.; Parra, J. R. P.; Zucchi, R. A.; Alves, S. B.; Vendramin, J. D.; Marchini, L. C.; Lopes, J. R. S.; Omoto, C. Entomologia agrícola. Piracicaba: FEALQ, 2002. 920p.

[10] Santos, H. R.; Nakano, O. Dados biológicos sobre a lagarta-rosca *Agrotis ipsilon* (Hufnagel, 1767) (Lepidoptera, Noctuidae). Anais da Sociedade Brasileira Entomológica do Brasil, v. 11, p. 33–48, 1982.

[11] Ávila, C. J.; Vivan, L. M.; Tomquelski, G. V. Ocorrência, aspectos biológicos, danos e estratégias de manejo de *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) nos sistemas de produção agrícolas. Circular Técnica 23, EMBRAPA, Dourados—MS, 2013.

[12] Galvão, A.; Attie, J.; Menezes, L.; Cunha, J.; Bisinotto, F. Relatório biotecnologia. Uberlândia: Céleres, 2012.

[13] Viana, P. A. Manejo de *Diabrotica speciosa* na cultura do milho. Circular Técnica 141.SeteLagoas, MG Setembro, 2010. 6p.

[14] Levine, E.; Oloumi-Sadeghi, H. Management of diabroticite rootworms in corn. Annual Review of Entomology, Palo Alto, v. 36, p. 229–255, 1991.

[15] Bravo, A.; Gill S. S.; Soberón M. *Bacillus thuringiensis* mechanisms and use. Comprehensive Molecular Insect Science Elsevier BV, p. 175–206, 2005.

[16] Bravo, A.; Gill, S. S.; Soberón, M. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon. v. 49, n. 4 p.423–435, 2007.

[17] Crickmore, N. et al. *Bacillus thuringiensis* toxin nomenclature. Disponível em: <http://www.lifesci.sussex.ac.uk/Home/Neil_Crickmore/Bt/>. Acessoem: 27 jan. 2015.

[18] Bravo, A.; Gómez, I.; Conde, J.; Muñoz-Garay, C.; Sánchez, J.; Miranda, R.; Zhuang, M.; Gill, S. S.; Soberón, M. Oligomerization triggers binding of a *Bacillus thuringiensis* Cry1Ab pore-forming toxin to aminopeptidase N receptor leading to insertion into membrane microdomains. Biochimica et Biophysica Acta, v. 1, p. 38–46, 2004.

[19] Carneiro, A. A.; Guimarães, C. T.; Valicente, F. H.; Waquil, J. M.; Vasconcelos, M. J. V.; Carneiro, N. P.; Mendes, S. M. Milho *Bt*: teoria e prática da produção de plantas transgênicas resistentes a insetos-praga. Sete Lagoas: Embrapa Milho e Sorgo, 2009. 25 p. (Embrapa Milho e Sorgo. Circular técnica, 135).

[20] CTNBio. Comissão Técnica Nacional de Biossegurança. Ministério da Ciência, Tecnologia e Inovação. 2015. Acesso em: 05/jun. Disponível: <http://www.ctnbio.gov.br/>.

[21] Bernardi, O.; Albernaz, K. C.; Valicente, F. H.; Omoto, C. Resistência de insetos-praga a plantas geneticamente modificadas. In: Borém, A.; Almeida, G. D. de. Plantas geneticamente modificadas: desafios e oportunidades para regiões tropicais. Visconde de Rio Branco: Suprema, 2011. p. 179–204.

[22] Martinelli, S.; Omoto, C. Resistência de insetos a plantas geneticamente modificadas. Biotecnologia,Ciência e Desenvolvimento, Brasília, DF, v. 34, p. 67–77, 2005.

[23] Huang, F.; Andow, D. A.; Buschman, L. L. Success of the high-dose/refuge resistance management strategy after 15 years of *Bt* crop use in North America. Entomologia Experimentalis et Applicata, Amsterdam, v. 140, n. 1, p. 1–16, 2011.

[24] Gassmann, A. J.; Petzold-Maxwell, J. L.; Keweshan, R. S.; Dunbar, M. W. Field-evolved resistance to *Bt* maize by Western Corn Rootworm. PLoS One, Cambridge, v. 6, n. 7, 2011.

[25] Kruger, M.; Van Rensburg, J. B. J.; Van Den Berg, J. Transgenic *Bt* maize: farmers' perceptions, refuge compliance and reports of stem borer resistance in South Africa. Journal of Applied Ecology, London, v. 136, n. 1–2, p. 38–50. 2012.

[26] Tabashnik, B. E.; Gassmann, A. J.; Crowder, D. W.; Carrière, Y. Insect resistance to *Bt* crops: evidence versus theory. Nature Biotechnology, London, v. 26, n. 2, p. 199–202, 2008.

[27] Matten, S. R.; Head, G. P.; Quemada, H. D. How governmental regulation can help or hinder the integration of *Bt*crops into IPM programs. In: Romeis, J.; Shelton, A. M.; Kennedy, G. G. Integration of insect resistant genetically modified crops within IPM program. New York: Springer, 2008. v. 5, p. 27–39.

[28] Storer, N. P.; Babcock, J. M.; Schlenz, M.; Meade, T.; Thompson, G. D.; Bing, J. W.; Huckaba, R. M. Discovery and characterization of field resistance to *Bt* maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. Journal of Economic Entomology, Lanham, v. 103, n. 4, p. 1031–1038, 2010.

[29] Villela, F. M. F.; Waquil, J. M.; Vilela, E. F.; Siegfried, B. D.; Foster, J. E. Selection of the fall armyworm, *Spodoptera frugiperda* (SMITH) (Lepidoptera: Noctuidae) for survival on Cry 1A(b) Bt toxin. Revista Brasileira de Milho e Sorgo, v. 1, n. 3, p. 12–17, 2002.

[30] Kumar, S.; Chandra, A.; Pande, K.C. *Bacillus thuringiensis* (Bt) transgenic crop: an environment friendly insect-pest management strategy. Journal of Environmental Biology, v. 29, p. 641–653, 2008.

[31] Smith, C. M.; Liu, X.; Wang, L. J.; Liu, X.; Chen, M.; Starkey, S.; Bai, J. Aphid feeding activates expression of a transcriptome of oxylipin-based defense signals in wheat involved in resistance to herbivory. Journal of Chemical Ecology, v. 36, p. 260–276, 2010.

[32] Wei, Z.; Hu, W.; Lin, Q.; Cheng, X.; Tong, M.; Zh U, L.; Chen, R.; He, G. Understanding rice plant resistance to the brown planthopper (*Nilaparvata lugens*): a proteomic approach. Proteomics, v. 9, p. 2798–2808, 2009.

[33] Chi, Y. H.; Sal, R. A. Z.; Balfe, S.; Ahn, J. E.; Sun, W.; Moon, J.; Yun, D. J.; Lee, S. Y.; Higg, T. J. V. I; Pittendr, B. I.; Murd, L. L. O.; Zh U-Sal Zman, K. Cowpea bruchid midgut transcriptome response to a soybean cystatin—costs and benefits of counter-defence. Insect Molecular Biology, v. 18, p. 97–110, 2009.

[34] Fu, Q.; Liu, P. C.; Wang, J. X.; Song, Q.S.; Zhao, X.F. Proteomic identification of differentially expressed and phosphorylated proteins in epidermis involved in larval-pupal metamorphosis of *Helicoverpa armigera*. BMC Genomics, v. 10, p. 600, 2009.

[35] Whiteman, N. K.; Groen, S. C.; Che Vasc O, D.; Bear, A.; Bec Kwith, N.; Greg Ory, T. R.; Den Oux, C.; Mammarella, N.; Ausubel, F. M.; Pierce, N. E. Mining the plant–herbivore interface with a leaf mining *Drosophila of Arabidopsis*. Molecular Ecology, v. 20, p. 995–1014, 2011.

[36] Matthes, M. C.; Bruce, T. J. A.; Ton, J.; Verrier, P. J.; Pickett, J. A.; Nap Ier, J. A. The transcriptome of cis-jasmone-induced resistance in *Arabidopsis thaliana* and its role in indirect defence. Planta, v. 232, p. 1163–1180, 2010.

[37] Broekgaarden, C.; Poelman, E. H.; Steenhui S, G.; Voorrips, R. E.; Dicke, M.; Vosman, B. Responses of *Brassica oleracea* cultivars to infestation by the aphid *Brevicoryne brassicae*: an ecological and molecular approach. Plant Cell and Environment, v. 31, p. 1592–1605, 2008.

[38] Li, Y.; Zou, J.; Li, M.; Bilgin, D. D.; Vodkin, L. O.; Hartman, G. L.; Clough, S. J. Soybean defense responses to the soybean aphid. New Phytologist, v. 179, p. 185–195, 2008.

[39] Ehlting, J.; Chowrira, S. G.; Mattheus, N.; Aeschliman, D. S.; Arimura, G. I.; Bohlmann, J. Comparative transcriptome analysis of *Arabidopsis thaliana* infested by dia-

mond back moth (*Plutella xylostella*) larvae reveals signatures of stress response, secondary metabolism, and signalling.BMC Genomics, v. 9, p. 20, 2008.

[40] Gilardoni, P. A.; Schuck, S.; Jüngling, R.; Rotter, B.; Baldwin, I. T.; Bonaventure, G. Super SAGE analysis of the *Nicotiana attenuate* transcriptome after fatty acid-amino acid elicitation (FAC): identification of early mediators of insect responses.BMC Plant Biology, v. 10, p. 66, 2010.

[41] Pauchet, Y.; Wilkinson, P.; Vogel, H.; Nelson, D. R.; Reynolds, S. E.; Heckel, D. G.; ffrench-Constant, R. H. Pyrosequencing the *Manduca sexta* larval midgut transcriptome: messages for digestion, detoxification and defence. Insect Molecular Biology, v. 19, p. 61–75, 2010.

[42] Hoffmann-Campo, C.B. Role of the flavonoids in the natural resistance of soyabean to *Heliothis virescens* (F.) and *Trichoplusia ni* (Hübner). 1995. 165 f. PhD dissertation, The University of Reading, Reading, 1995.

[43] Piubelli, G. C.; Hoffmann-Campo, C. B.; Moscardi, F.; Miyakubo, S. H.; Oliveira, M.C.N. Are chemical compounds important for soybean resistance to *Anticarsia gemmatalis.* Journal of Chemical Ecology, v. 31, p. 1515–1531, 2005.

[44] Piubelli, G.C. Bioatividade de genótipos de soja resistentes a *A. gemmatalis* Hübner (Lepidoptera: Noctuidae) e interações de suas substâncias químicascom inimigos naturais. 2004. 126f. Tese (Doutorado)—Universidade Federaldo Paraná, Curitiba, 2004.

[45] Hoffmann-Campo, C.B.; Harborne, J.B.; Miccafferry, A.R. Préingestiveand post-ingestive effects of soya bean extracts and rutin on *Trichoplusia ni* growth. Entomologia Experimentalis et Applicata, v. 98, p. 181–194, 2001.

[46] Bellés, X. Beyond *Drosophila*: RNAi in vivo and functional genomics in insects. Annual Review of Entomology, v. 55, p. 111–28, 2010.